7

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

WASHINGTON, DC 20460

OPP OFFICIAL RECORD
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

OFFICE OF PREVENTION, PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

DATE: September 6, 2007

SUBJECT: Beta-Cyfluthrin: Developmental Neurotoxicity Study in Rats.
PC Code: 118831 DP Barcode: D293830 TXR #: 0052372

TO: George LaRocca, PM 13
Insecticides Branch
Registration Division (7505P)

FROM: Alan C. Levy, Physiologist Alan C. Levy
Registration Action Branch 2
Health Effects Division (7509P)

THRU: Richard Loranger, Branch Senior Scientist for
Registration Action Branch 2
Health Effects Division (7509P)

ACTION REQUESTED: The Health Effects Division (HED) was asked to review the submitted developmental neurotoxicity study in rats conducted with beta-cyfluthrin (MRID 46054101).

CONCLUSIONS: The Data Evaluation Record for this study is attached. The developmental neurotoxicity study in rats was classified acceptable/non-guideline.

Rec'd in RAC 3/18/2008 EW

DATA EVALUATION RECORD

BETA CYFLUTHRIN

STUDY TYPE: DEVELOPMENTAL NEUROTOXICITY STUDY - RAT; OPPTS 870.6300

MRID 46054101

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Toxicology and Hazard Assessment Group
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task No. 21-2004

Primary Reviewer:
Cheryl B. Bast, Ph.D., D.A.B.T

Signature: Cheryl B Bast
Date: FEB 28 2008

Secondary Reviewers:
Carol Wood, Ph.D., D.A.B.T.

Signature: Carol Wood
Date: FEB 28 2008

Robert H. Ross, M.S. Group Leader

Signature: Robert H. Ross
Date: FEB 28 2008

Quality Assurance:
Lee Ann Wilson, M.A.

Signature: L. A. Wilson
Date: FEB 28 2008

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory is managed and operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

EPA Reviewer: Alan C. Levy, Ph.D. **Signature:** Alan C. Levy
Registration Action Branch 2, Health Effects Division (7509P) **Date:** 3-4-2008
Work Assignment Manager: G. Dannan, Ph.D. **Signature:**
Registration Action Branch 3, Health Effects Division (7509P) **Date:** 3/6/08

TXR#: 0052372

DATA EVALUATION RECORD

STUDY TYPE: Developmental Neurotoxicity Study - Rat; OPPTS 870.6300 (§83-6); OECD 426

PC CODE: 118831 **DP BARCODE:** D293830

TEST MATERIAL (PURITY): Technical Grade β-Cyfluthrin (95.1-97.6%)

SYNONYMS: FCR 4545 Technical; Cyano(4-fluoro-3-phenoxyphenyl)methyl-3-(2,2-dichloroethenyl)-2,2-dimethyl-cyclopropanecarboxylate

CITATION: Sheets, L. P. (2003) A Developmental neurotoxicity screening study with technical grade β-Cyfluthrin in Wistar rats. Bayer CropScience LP, 17745 South Metcalf Ave., Stilwell, Kansas, 66085-9104. Laboratory report number 200620; July 29, 2003. MRID 46054101. Unpublished.

SPONSOR: Bayer CropScience LP, 2 T. W. Alexander Drive, Research Triangle Park, NC 27709.

EXECUTIVE SUMMARY: In a developmental neurotoxicity study (MRID 46054101), β-Cyfluthrin (95.1-97.6% a.i., batch #8030130) was administered to 30 parent female Wistar rats/dose in the diet at nominal concentrations of 0, 30, 125, or 200 ppm from gestation day 0 through postnatal day 21. The average daily intake of β-Cyfluthrin was 0, 2.4, 11.0, and 17.8 mg/kg/day during gestation and 0, 5.9, 25.4, and 40.9 mg/kg/day during lactation for the 0, 30, 125, and 200 ppm groups, respectively. A Functional Observational Battery (FOB) was performed on 30 dams/dose on gestation days 6 and 20 and on 10 dams/dose on lactation days 11 and 21. On postnatal day 4, litters were culled to yield four males and four females (as closely as possible). Offspring representing at least 20 litters/dose were allocated for detailed clinical observations (abbreviated FOB), assessment of motor activity, assessment of auditory startle response habituation, assessment of learning and memory, and opthalmology. Neural tissues were collected from 10 offspring/sex/dose (representing 20 litters) on PND 21 (brain only) and at study termination (day 75 of age) for micropathologic examination and morphometric analysis. The concentration of β-Cyfluthrin in the whole brain from dams (PND 21) and offspring (PND 4 and PND 21) was also measured to verify exposure. Pup physical development was assessed by body weight, and sexual maturation of females was assessed by age at vaginal opening. Maturation of males was assessed by age at completion of balano-preputial separation.

In dams, no treatment-related effects were seen on mortality, clinical signs, body weight, body weight gain, or reproductive performances. The decreases in the body weights of dams at the

3

high dose during the first week of treatment and lactation days 4, 7 and 14 were not considered to be adverse because they were transient, the decrease was of low magnitude (7-8%), occurred in the absence of any effect on food consumption, and rebounded on LD21. **The maternal NOAEL is 17.8 mg/kg/day, the highest dose tested. A maternal LOAEL was not established.**

In offspring, treatment had no adverse effects on survival, clinical signs, birth weight, developmental landmarks, FOB parameters, motor or locomotor activity, auditory startle reflex, learning and memory, brain morphology or neuropathology. Body weight and body weight gain were comparable across all dose groups at birth and on PND 4. Body weight was decreased (8-11%) in high-dose (200 ppm, 17.8 mg/kg/day) pups on subsequent days through weaning. Weight gain of high-dose pups was decreased 8.5%-13% from PND 4-21. After weaning, pups received only untreated diet, and the only compound-related effects were noted in high-dose males and females and were associated with the decreased body weight that developed during lactation. High-dose males weighed 10% less than controls during the first week post-weaning and 7% less during the last week of the study. High-dose females weighed 7.5% less than controls during the first week post-weaning and had weight comparable to controls by the last two weeks of the study. At termination, absolute brain weight was decreased 5.8% ($p \leq 0.05$) in high-dose females compared to controls.

The offspring LOAEL is 17.8 mg/kg/day based on decreased body weight and body weight gain and decreased brain weights in females at termination. The offspring NOAEL is 11.0 mg/kg/day.

This study is classified **Acceptable/non-guideline** and may be used for regulatory purposes; however it does not satisfy the guideline requirement for a developmental neurotoxicity study in rats (OPPTS 870.6300, §83-6; OECD 426 (draft)) at this time pending a comprehensive review of all available positive control data.

COMPLIANCE: Signed and dated Flagging, GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. **Test material:** Technical grade β-Cyfluthrin

Description:	Off-white powder
Lot/Batch #:	8030130
Purity:	95.1-97.6 % a.i.
Compound Stability:	Confirmed for 28 days in diet, frozen; confirmed for 7 days in diet, at room temperature
CAS # of TGAI:	68359-37-5

2. **Vehicle and/or positive control:** acetone solvent in the diet

3. **Test animals (P):**

Species:	Rat	
Strain:	Wistar Hannover Crl:WI(Glx/BRL/Han) IGS BR	
Age at study initiation:	females: at least 12 wks; males: at least 15 weeks (breeders only)	
Wt. at study initiation:	173.6-237.0 g	
Source:	Charles River Laboratories, Raleigh, NC	
Housing:	Individually or with litter in stainless steel grid or plastic cages	
Diet:	Purina Mills Rodent Lab Chow 5002, *ad libitum*	
Water:	Tap water, *ad libitum*	
Environmental conditions:	**Temperature:**	19-25°C
	Humidity:	30-70%
	Air changes:	10-15/hour
	Photoperiod:	12 hrs dark/12 hrs light
Acclimation period:	At least 6 days	

B. PROCEDURES AND STUDY DESIGN:

1. **In life dates**: Start: January 14, 2002; End: April 18, 2002

2. **Study schedule:** The maternal animals were mated and assigned to the study. The test substance was administered to the maternal animals (30/dose group) from gestation day 0 through lactation day 21. Pups were weaned onto control diets on postnatal day 21, after which time maternal animals were killed. F_1 pups remained on study up to postnatal days 70-80.

3. **Mating procedure:** Females were paired 1:1 with males of the same strain and source. Each female was examined daily during the mating period to identify sperm cells in a vaginal smear or the presence of a copulatory plug. The day that sperm or a plug was found was designated gestation day 0. After successful mating, each pregnant female was placed into

an individual cage with a solid bottom and bedding, where the dam was maintained through gestation and lactation.

4. **Animal assignment:** Mated females and offspring were allocated as shown in Table 1 using an animal allocation program written in SAS. For offspring, four sets of animals (designated sets A-D) were utilized for assessment at each age. Randomly-selected pups (10/sex/dose) were designated as Set D and were per fused with fixative and brains were collected for histopathological examination and morphometric analysis.

   Sixteen pups/sex/group were allocated on postnatal day 4 to each of the following: motor activity, acoustic startle habituation, passive avoidance, water maze, detailed observational battery, and sacrifice and brain examination on postnatal day 21. At approximately 50-60 days of age, a minimum of 10 offspring/sex/dose level were given an opthalmoscopic examination. On day 70-80, animals were sacrificed by perfusion and brain weight recorded. The concentration of β-Cyfluthrin in the whole brain from dams (PND 21) and offspring (PND 4 and PND 21) was also measured to verify exposure.

TABLE 1. Study design

Experimental parameter		Dose (ppm in diet) 0	30	125	200
Maternal animals					
		No. of maternal animals assigned			
FOB (GD 6,20)		30	30	30	30
FOB (LD 11, 21)		10	10	10	10
Concentration of β-Cyfluthrin in whole brain (LD 21)		18-22	18-22	18-22	18-22
Offspring					
Set A	Motor activity (PND 13, 17, 21, 58-62)	16/sex	16/sex	16/sex	16/sex
Set B	Acoustic startle habituation (PND 22, 36-40, 58-62)	16/sex	16/sex	16/sex	16/sex
Set C	Passive avoidance (PND 22, 29)	16/sex	16/sex	16/sex	16/sex
	Detailed clinical/FOB (PND 4, 11, 21, 35, 45, 60)	16/sex	16/sex	16/sex	16/sex
	Water maze (PND 58-62, 7 days after first test)	16/sex	16/sex	16/sex	16/sex
Sets A-C	Opthalmologic evaluation (PND 50-60)	10/sex	10/sex	10/sex	10/sex
	Brain Weight (PND 70-80)	10/sex	10/sex	10/sex	10/sex
Set D	Gross necropsy, Concentration of β-Cyfluthrin in whole brain, and brain measurements (PND 21)*	10/sex	10/sex	10/sex	10/sex

*Page 23 of MRID 46054101 states that approval was granted by OPPTS/OPP/HED staff to replace PND 11 with PND 21 for neuropathology for this study.

5. **Dose selection rationale:** Dose levels were chosen based on the results from a 90-day neurotoxicity study in F344 rats (Bayer Report 107491; MRID 44296001) and a two-generation reproduction study in Sprague-Dawley rats (Bayer Report 107408; MRID 44371401). In the neurotoxicity study, β-Cyfluthrin was administered in the diet at concentrations of 0, 30, 125, or 400 ppm for 13 weeks. No treatment-related effects were noted in low-concentration animals. Males and females in the 125 ppm group exhibited

dermal lesions around the head, and females exhibited decreased body weight (8% decrease, weeks 5-13) and food consumption. In high-concentration animals, ataxia, repetitive chewing or pawing, decreased grip strength, and decreased body weight (17% decrease throughout study) were noted in both sexes. Decreased body temperature and incoordinated aerial righting were observed in 400 ppm females.

In the two-generation reproduction study, β-Cyfluthrin was administered in the diet at levels of 0, 50, 125, or 400 ppm beginning 10 weeks before mating. There were no treatment-related effects in dams or offspring in the 50 ppm group. Decreased body weight in dams during gestation (11% decrease; GD 0-20) and in offspring during lactation (6-7% decrease LD 7 and 14) were noted in the 125 ppm group. Decreased body weight in dams during gestation (13% decrease; GD 0-20) and in offspring during lactation (7-14% decrease LD 7 and 14) were also noted in the 400 ppm group. High-dose dams exhibited hind limb splay during lactation, and high-dose offspring showed decreased body weight (8% decrease on PND 4 and 20% decrease on PND 21) and course tremors during lactation. There were no other compound-related findings.

Based on the results of these studies, the doses selected for the developmental neurotoxicity study were 0, 30, 125, and 200 ppm. The 200 ppm level was selected to produce overt maternal toxicity and evidence of toxicity in the offspring. The 30 ppm level was selected to be an overall NOAEL in the pups, with possible slight effects in the dams. The 125 ppm dose level was selected as an intermediate dose to assist in establishing compound-related effects.

6. **Dosage administration:** β-Cyfluthrin was administered to parent female Wistar rats in the diet at levels of 0, 30, 125 or 200 ppm from gestation day 0 through postnatal day 21. The test substance intake was 0, 2.4, 11.0, or 17.8 mg/kg/day, respectively, for analytically-determined concentrations of 0, 29.0, 133, or 215 ppm in the diet during gestation. The test substance intake was 0, 5.9, 25.4, or 40.9 mg/kg/day, respectively, for analytically-determined concentrations of 0, 29,133, or 215 ppm in the diet during lactation.

7. **Dosage preparation and analysis:** Detailed descriptions of feed preparations and test diet analysis were not provided; however, information from the study report is as follows: Acetone was used as the solvent to dissolve the test article for mixing in the diet and was allowed to evaporate before the feed was given to the animals. The control diet was similarly prepared, excluding the test substance. Concentrations of the test substance in the diet were measured by liquid chromatography four times (weeks 1, 2, 3, and 6) during the in-life phase of the study. Homogeneity of β-cyfluthrin in the diet was determined using feed mixed for week one of the study (utilizing concentrations of 30 and 200 ppm), and stability data were determined by analyzing 30 and 200 ppm diets on days 0, 7, 14, and 28 under freezer storage conditions and on days 0 and 7 at room temperature.

Results:

Homogeneity analysis: was determined from nine samples of ration from each level of the 30 and 200 ppm diets. The mean concentrations were 28.3 ± 2.79 ppm (CV=9.9%) for the 30 ppm diet and 217 ± 8.03 ppm (CV=3.7%) for the 200 ppm test diet.

Stability analysis: At nominal concentrations of 30 ppm and 200 ppm, β-cyfluthrin is stable in the diet for at least 7 days at room temperature (Day 7: 30 ppm diet 107% of initial; 200 ppm diet 113% of initial) and 28 days at freezer conditions (Days 7-28: 30 ppm diet 105-88.5% of initial; 200 ppm diet 113-109% of initial).

Concentration analysis: The 30, 125, and 200 ppm dietary levels averaged 96.7%, 106%, and 108% of the nominal concentration, respectively.

The analytical data indicated that the concentration, stability, and homogeneity of β-cyfluthrin in the diets were adequate.

C. OBSERVATIONS:

1. In-life observations:

a. **Maternal animals:** Once daily checks for mortality or moribundity and daily cage-side observations were conducted for maternal animals.

Thirty dams per group were observed (by observers blind to the treatment group) outside the home cage during the gestation dosing period (days 6 and 20) and at least 10 dams/group were observed during the lactation dosing period (days 11 and 21). The following functional observations were recorded.

	Functional observations–Maternal animals
X	Signs of autonomic function, including: 1) Ranking of degree of lacrimation and salivation, with range of severity scores from none to severe 2) Presence or absence of piloerection and exophthalamus, 3) Ranking or count of urination and defecation, including polyuria and diarrhea 4) Pupillary function such as constriction of the pupil in response to light, or a measure of pupil size 5) Degree of palpebral closure, e.g., ptosis.
X	Description, incidence, and severity of any convulsions, tremors, or abnormal movements.
X	Description and incidence of posture and gait abnormalities.
X	Description and incidence of any unusual or abnormal behaviors, excessive or repetitive actions (stereotypes), emaciation, dehydration, hypotonia or hypertonia, altered fur appearance, red or crusty deposits around the eyes, nose, or mouth, and any other observations that may facilitate interpretation of the data.

Individual maternal body weight and food consumption data were recorded on gestation days 0, 6, 13, and 20, and lactation days 0, 4, 7, 14, and 21. Food consumption measurements may have included consumption by the pups, especially during lactation week 3.

From gestation day 20, dams were checked daily for evidence of parturition. They were permitted to deliver and rear offspring until postnatal day 21. Numbers of live and dead offspring were recorded during parturition.

Selected pups from Set D and their mothers were used for body temperature measurements. A microchip was implanted subcutaneously in the region between the scapulae on PND 8,

and temperatures were measured by telemetry on days 10, 15, 18, and 21 postpartum. Temperatures were measured early in the morning, and observations were recorded to establish whether pups were with litter mates, the dam, or were isolated; activity level was also recorded.

b. Offspring:

1. **Litter observations:** Daily throughout lactation, offspring were examined cage-side for gross signs of mortality or morbidity.

   On day 4 postpartum, litters were standardized to a maximum of 8 pups/litter (4/sex/litter, as nearly as possible); excess pups were killed and discarded. If there were more than 23 acceptable litters for any dietary level, the surplus litters were weighed and sacrificed without routine necropsy on PND 4.

2. **Developmental landmarks:** Beginning on postnatal day 38, male offspring were examined daily for balanopreputial separation. Beginning on postnatal day 29, female offspring were examined daily for vaginal patency. The age of onset was recorded.

3. **Detailed observations:** Offspring were examined for clinical signs once daily during the preweaning period and once weekly after weaning by observers who were aware of the treatment groups. Individual offspring body weight data were recorded on postnatal days 0, 4, 11, 17, and 21 and once weekly thereafter. Individual food consumption was measured weekly from the week of postnatal day 28.

4. **Neurobehavioral evaluations:** Observations and the schedule for those observations are summarized as follows from the report.

5. **Functional observational battery (FOB):** On postnatal days 4, 11, 21, 35, 45, and 60, a total of 16 offspring/sex/group (one male or one female from each litter) were examined outside the home cage in an FOB assessment by observers blind to the treatment groups. On postnatal days 4 and 11, the animals were not evaluated in the open field, unless deemed necessary by the observer. Otherwise, methods were similar to the procedures used for the dams. Selected pups from Set D and their mothers were used for body temperature measurements. A microchip was implanted subcutaneously in the region between the scapulae on PND 8, and temperatures were measured by telemetry on days 10, 15, 18, and 21 postpartum. Temperatures were measured early in the morning, and observations were recorded to establish whether pups were with litter mates, the dam, or were isolated; activity level was also recorded.

	FUNCTIONAL OBSERVATIONS- Offspring
X	Signs of autonomic function, including: 1) Ranking of degree of lacrimation and salivation, with range of severity scores from none to severe 2) Presence or absence of piloerection and exophthalamus, 3) Ranking or count of urination and defecation, including polyuria and diarrhea 4) Pupillary function such as constriction of the pupil in response to light, or a measure of pupil size 5) Degree of palpebral closure, e.g., ptosis.
X	Description, incidence, and severity of any convulsions, tremors, or abnormal movements.
X	Description and incidence of posture and gait abnormalities.
X	Description and incidence of any unusual or abnormal behaviors, excessive or repetitive actions (stereotypies), emaciation, dehydration, hypotonia or hypertonia, altered fur appearance, red or crusty deposits around the eyes, nose, or mouth, and any other observations that may facilitate interpretation of the data.

6. **Motor activity testing:** Motor activity was evaluated in 16 rats/sex/dose on days 13, 17, 21, and 60. Animals were placed individually in figure-eight mazes and were continuously monitored over a 1-hour period. An automated activity monitoring system collected data over successive 10-minute intervals by recording infra-red light source break frequency within the maze. Motor activity was measured as the number of beam interruptions that occurred during the test session, and locomotor activity was measured by eliminating consecutive counts for a given beam. Therefore, only one interruption of a given beam was counted for locomotor activity until the rat relocated in the maze and interrupted a different beam. Habituation was evaluated as a decrease in activity over consecutive test-session intervals.

7. **Auditory startle habituation:** Auditory startle reflex habituation was performed on 16 offspring/sex/dose on postnatal days 22, 38 and 60, using an automated system.

   Animals were acclimated for 5 minutes to background noise and were then presented with the startle stimulus at 10-second intervals. The startle stimulus consisted of 50-millisecond bursts of white noise at approximately118 dB. Peak response amplitude (g force exerted on the platform) and latency (msec) measurements were recorded for each animal's individual response curve. Response amplitude was defined as the maximum value of the average curve minus the baseline (body weight). Latency to peak was defined as the time, in msec, following onset of the stimulus when the peak response amplitude occurred.

8. **Learning and memory testing:**

   PASSIVE AVOIDANCE CONDITIONING: On postnatal days 22 and 29, learning and short- and long-term retention were assessed in a passive avoidance test of 16 offspring/sex/dose. Testing was done in individual isolation cubicles each with a single shuttle cage. Each cubicle was insulated to attenuate sound and had a fan for ventilation. Each 7 x 7 inch shuttle cage was separated into two equal-sized compartments by a centrally-located sliding door. The two compartments were identical except that the walls in one compartment were lined with black film (dark side), and the walls in the other compartment were not lined and this compartment was illuminated with a high-intensity lamp. The lamp

was switched on at the beginning of each trial and remained on until the rat crossed into the dark compartment or the trial ended. The cage floor was constructed of a stainless steel grid and the movement of the rat from the light to dark side was detected by a photocell. Rats were placed individually into the shuttle cage facing toward the light. After 20 seconds, the light was switched on and the door separating the compartments was opened. When the rat crossed into the dark side, the door closed, a brief, mild shock (0.5 sec, 0.5mA) was delivered, and the light was switched off. If the rat failed to cross to the dark side within 180 seconds, it was returned to the holding cage and assigned a latency time of 180 sec. The procedure was repeated until the rat either remained in the bright side for 180 seconds for two consecutive trials or until 15 trials had elapsed (whichever occurred first). Rats that failed to reach criterion performance within 15 trials or failed to cross during the first two acquistion trials were excluded from the retention phase of the experiment.

WATER MAZE: Learning and memory testing were performed in 16 offspring/sex/dose on postnatal days 60 and again seven days later using an M-water maze. Only rats that demonstrated acquisition on the first test occasion were tested for retention seven days later. The water maze was made of opaque Plexiglas with 5-inch wide corridors. The walls were 16-inches high with approximately 7.5 inches of water. The maze was filled with water at 22±1°C. For each test trial, the rat was placed at the base of the M-maze stem, between the two lateral arms. On the learning trial (first trial), the rat was required to enter both arms of the maze before being provided access to the exit ramp to escape the water and was then removed from the maze. The initial arm chosen on the learning trial was designated the incorrect goal during the subsequent trials (15 maximum). Rats failing to make a correct goal choice within 60-seconds in any given trial were led to the correct goal with the exit ramp and then removed from the water. The inter-trial interval was approximately 15 seconds. Each rat was required to reach a criterion of 5 consecutive error-free trials to stop the test session. Latency (in seconds) to choose the correct goal or the maximum 60-second interval was recorded for each trial, as well as the number of errors (incorrect turns) during each trial.

9. Ophthalmology:

At postnatal days 50-60, indirect ophtalmoscopy was performed on 10 offspring/sex/dose (that had been selected for perfusion) following dilation with a mydriatic agent.

D. Postmortem observations:

Brain concentrations of β-cyfluthrin:

The whole brain was collected from culled pups (PND 4) and from selected Set D pups and their mothers on PND 21 for measurement of β-cyfluthrin concentrations in order to confirm exposure. β-cyfluthrin residues were extracted from frozen brain tissues by accelerated solvent extraction, using acetonitrile at 50°C and 1500 PSI. Extracts of the tissues were then purified through C-18 solid phase extraction cartridges. The resultant analyte (cyano(4-fluoro-3-phenoxyphenyl)methyl 3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylate) was then determined by GC/MS.

1. **Maternal animals:** Maternal animals were sacrificed by carbon dioxide inhalation on postnatal day 21. Adult females were not routinely subjected to gross necropsy. Maternal animals found moribund were sacrificed. Those found moribund or dead were subjected to macroscopic necropsy, with possible collection of tissues at the discretion of the study director.

2. **Offspring:** The offspring selected for brain weight or neuropathological evaluation were sacrificed on postnatal day 21 or 70-80. These animals were subjected to postmortem examinations as described below:

   At postnatal day 21, up to 10 pups/sex/group were sacrificed by intraperitoneal injection of pentobarbital (50 mg/kg) and perfused via the left ventricle with a sodium nitrite flush followed by fixation with 1% gluteraldehyde and 4% paraformaldehyde. The brain was collected, weighed, and post-fixed with 10% buffered formalin. Anterior to posterior cerebrum and cerebellum lengths were measured by an individual not blind to treatment using a Vernier caliper. Brains from all dose groups were embedded in paraffin with sections being made from control and high-dose animals. Tissues were sectioned at 5 μm and stained with hematoxylin and eosin and luxol fast blue/cresyl violet. Eight coronal sections from control and high-dose animals were examined microscopically.

   The following brain morphometric measurements were performed:
   Frontal cortex thickness (dorsal portion of the cerebral cortex within the coronal section passing through the region of the optic chiasm)

   Parietal cortex thickness (dorsolateral portion of the cerebral cortex within the coronal section taken through the optic chiasm)

   Caudate putamen horizontal width (coronal section taken at the level of the optic chiasm)

   Corpus callosum (thickness at the midline)

   Hippocampal gyrus (greatest dorsal-ventral thickness)
   Cerebellum (roof of the fourth ventricle to the dorsal surface)

   On postnatal day 70-80, 10 animals/sex/group were euthanized by carbon dioxide asphyxiation, underwent a gross necropsy and the brains were removed and weighed (fresh weight); the animals were then discarded. Another 10 rats/sex/dose were sacrificed by intraperitoneal injection of pentobarbital (50 mg/kg) and perfused via the left ventricle with a sodium nitrite flush followed by fixation with 1% gluteraldehyde and 4% paraformaldehyde. The brain, spinal cord, both eyes with optic nerves, peripheral nerves, gasserian ganglion, gastrocnemius muscle, and both forelimbs were collected, weighed (brain only), and post-fixed with 10% buffered formalin. Anterior to posterior cerebrum and cerebellum lengths were measured by an individual not blind to treatment using a Vernier caliper.

   The following central and peripheral nervous system tissues were dissected and preserved in paraffin (CNS tissues) or plastic (PNS tissues): eight coronal sections of the brain, cervical, thoracic, and lumbar sections of the spinal cord, the cauda equina, eyes, optic nerves,

gastrocnemius muscle, dorsal root ganglia and fibers, and gasserian ganglion. Tissues from all dose groups were embedded; however, only control and high-dose tissues were examined unless effects warranted examination of low- and mid-dose samples. Paraffin-embedded tissues were sectioned at 5 μm and stained with hematoxylin and eosin. Plastic-embedded tissues were sectioned at 2-3 μm and stained with a modified Lee's stain.

Detailed morphometric evaluation of the neocortex, hippocampus, and cerebellum was conducted as follows:

Frontal cortex thickness (dorsal portion of the cerebral cortex within the coronal section passing through the region of the optic chiasm)

Parietal cortex thickness (dorsolateral portion of the cerebral cortex within the coronal section taken through the optic chiasm)

Caudate putamen horizontal width (coronal section taken at the level of the optic chiasm)

Corpus callosum (thickness at the midline)

Hippocampal gyrus (greatest dorsal-ventral thickness)

Cerebellum (roof of the fourth ventricle to the dorsal surface)

E. DATA ANALYSIS:

1. **Statistical analyses:** Continuous data were initially analyzed for equality of variance using Bartlett's test. Group means with equal variances were further analyzed with ANOVA, followed by Dunnett's test if significance was identified with the ANOVA. Group means with unequal variances were analyzed by Kruskal-Wallis ANOVA followed by the Mann-Whitney U test for between-group comparisons. The level of significance was set at $p \leq 0.05$, except for Bartlett's test which was set at $p \leq 0.001$.

   Motor and locomotor activity were analyzed with ANOVA, followed by Dunnett's test if significance was attained with ANOVA. Acoustic startle peak amplitude data were analyzed by ANOVA followed by Dunnett's test if significance was observed with the ANOVA. The response amplitude data for each block of 10 trials were subjected to a Repeated-Measures ANOVA, using the test block as the repeated measure. Passive avoidance latency data were analyzed with a Wilcoxon Test for time to failure. The number of trials to criterion was analyzed with Kruskal-Wallis and Wilcoxon tests for the acquisition phase and Fisher's exact test for retention. The number of rats failing to meet the criterion level of performance in the acquisition phase was treated as incidence data. Water maze data were analyzed by a univariate ANOVA followed by Dunnett's test. The number of trials to criterion and the number of errors were analyzed with Kruskal-Wallace and Wilcoxon test for the acquisition phase and Fischer's exact test for retention. The number of rats failing to meet the criterion level of performance in the water maze learning phase was treated as incidence data. Micropathology frequency data were analyzed by Chi-Square followed by Fisher's Exact Test if significance was identified with the Chi-Square.

2. **Indices:**

   a. **Reproductive indices:** The following reproductive indices were calculated from breeding and parturition records of animals in the study:

   Mating index = (Number of inseminated females/Number of females co-housed with males) × 100

   Fertility index = (Number of pregnant females/Number of inseminated females) × 100

   b. **Offspring viability indices:** The following viability (survival) indices were calculated from lactation records of litters in the study:

   Live birth index = (Number of live offspring per litter /Total number of offspring born per litter) × 100

   Viability index = (Number of live offspring at PND 4 per litter/Number of live offspring born per litter) × 100

   Lactation index = (Number of live offspring on Day 21 per litter/Number of live offspring on PND 4 after culling per litter) × 100

3. **Positive and historical control data:** Positive control/methodology validation and historical control studies have been submitted to the Agency (MRID Nos. 45441301, 45441302, 45441303 and 45464602).

II. RESULTS:

A. PARENTAL ANIMALS:

1. **Mortality and clinical and functional observations:** No dams were found dead or were sacrificed in moribund condition during gestation or lactation. There were no treatment-related clinical signs observed during gestation or lactation. The only observations during gestation were red lacrimal staining in one high-dose female and alopecia in one mid-dose and two high-dose dams. The incidences of the observations are within historical control ranges, and the alopecia is associated with dams preparing for delivery. Thus, the effects are considered incidental to treatment.

2. **Body weight and food consumption:** Selected group mean body weight and food consumption values for pregnant or nursing dams are summarized in Table 2. Body weight was decreased 7% ($p \leq 0.01$) in high-dose females during the first week of exposure; however, there were no differences in the subsequent weeks of gestation. During lactation, body weight was decreased in high-dose dams on LD 4 (8%), LD 7 (6%), and LD 14 (7%), but was comparable to controls on LD 21. There were no other treatment-related effects on body weight or body weight gain during gestation or lactation. There were no treatment-related

effects on food consumption during gestation. During the third week of lactation, high-dose dams consumed 10% less food than controls. No other effects were noted.

TABLE 2. Selected mean (±SD) maternal body weight and food consumption[a]

Observations/study interval	Dose (ppm)			
	0	30	125	200
Gestation (n=29-30)				
Body wt. Gestation day 0 (g)	206.0±2.27	201.9±3.09	203.6±2.76	200.3±2.38
Body wt. Gestation day 6 (g)	224.9±2.27	222.5±3.06	222.3±2.72	208.6**±4.75 (7%)
Body wt. Gestation day 13 (g)	247.8±2.72	246.0±3.40	249.0±2.94	238.6±3.37
Body wt. Gestation day 20 (g)	299.2±6.24	302.5±5.21	306.0±4.51	294.7±5.60
Wt. gain gestation days 0-20 (g)	93.1±5.81	100.7±4.61	102.4±3.63	94.5±4.87
Food consumption gestation days 0-6 (g/day)	15.6±0.29	15.8±0.48	16.3±0.35	15.5±1.05
Food consumption gestation days 6-13 (g/day)	20.2±1.18	19.3±0.89	18.7±0.43	18.0±0.41
Food consumption gestation days 13-20 (g/day)	20.1±0.67	20.7±0.57	20.7±0.45	19.9±0.51
Lactation (n=18-29)				
Body wt. lactation day 0(g)	243.4±2.47	239.5±3.38	241.5±3.32	231.5±3.61
Body wt. lactation day 4 (g)	257.9±3.14	251.4±3.18	249.0±4.36	238.3**±3.36 (8%)
Body wt. lactation day 7 (g)	266.2±3.01	259.9±3.93	259.3±3.67	250.3**±3.40 (6%)
Body wt. lactation day 14(g)	287.0±3.38	279.4±3.87	278.9±3.59	267.3**±3.98 (7%)
Body wt. lactation day 21(g)	275.3±3.72	274.5±4.22	274.7±3.67	268.2±3.79
Food consumption lactation days 0-7 (g/day)	34.7±1.25	41.1±4.44	34.3±2.56	32.8±1.32
Food consumption lactation days 7-14 (g/day)	53.8±1.48	52.1±1.67	50.6±1.14	49.1±1.36
Food consumption lactation days 14-21 (g/day)	69.5±2.09	67.0±1.49	65.1±1.36	62.3*±1.19 (10%)

[a]Data obtained from Tables 3 & 4 pages 59-62 and Tables 6 & 7 pages 65-68, MRID 46054101. *p≤ 0.05, **p≤0.01. Number in parentheses is % difference compared to control.

15

3. **Reproductive performance:** There were no treatment-related effects on fertility, gestation indices or gestation length. Results for the maternal animals are summarized in Table 3.

TABLE 3. Reproductive Performance [a]

Observation	Dose (ppm)			
	0	30	125	200
Number Mated	30	30	30	30
Mating Index (%)	100.0	100.0	100.0	100.0
Fertility Index (%)	86.7	96.7	96.7	86.7

[a]Data obtained from Table 1, pages 56-57, MRID 46054101.

4. **Compound intake:** Compound intake during gestation and lactation is summarized in Table 4.

TABLE 4. Compound Intake (mg/kg/day) [a]

Gestation	Dose (ppm)			
	0	30	125	200
Day 0-6	0.0 ± 0.00	2.3 ± 0.07	10.7 ± 0.23	16.6 ± 1.06
Day 6-13	0.0 ± 0.00	2.5 ± 0.11	11.2 ± 0.25	18.7 ± 0.41
Day 13-20	0.0 ± 0.00	2.5 ± 0.06	11.1 ± 0.19	18.0 ± 0.39
Lactation				
Day 0-7	0.0 ± 0.00	5.0 ± 0.61	19.0 ± 1.45	30.6 ± 1.44
Day 7-14	0.0 ± 0.00	5.8 ± 0.18	26.0 ± 0.39	42.3 ± 1.35
Day 14-21	0.0 ± 0.00	7.0 ± 0.17	31.2 ± 0.54	49.8 ± 0.55

Data obtained from pp. 70 & 71, MRID 46054101.

5. **Maternal postmortem results:** β-Cyfluthrin was detected in a dose-related manner in brain tissue from dams in all treatment groups on LD 21. Data are summarized below.

Dose group (ppm)	Concentration (ppm) in whole brain tissue
0	0.000
30	0.006
125	0.026
200	0.046

Data obtained from page 49, MRID 46054101.

B. OFFSPRING:

1. **Viability and clinical signs:** Litter size and viability (survival) results from pups during lactation are summarized in Table 5. There was no treatment-related effect on the number of litters, live litter size, number of stillborn pups, live birth index, or viability index.

TABLE 5. Litter size and viability [a]

Observation	Dose (ppm)			
	0	30	125	200
Number of Litters*	19	23	23	23
Total number born	220	260	254	271
Number born live	219	260	250	269
Number born dead	1	0	4	2
Mean No. of viable pups:				
Day 0	12	11	11	12
Day 4 [b]	11	11	11	12
Day 4 [c]	8	8	8	8
Day 21	8	8	8	8
Live birth index (%)	99.4	100	99.6	99.0
Viability index	98.6	99.3	99.3	99.6
Lactation index	100	100	98.9	98.9

[a]Data obtained from Table 9, pages 72-74, MRID 46054101.
[b]Before standardization (culling).
[c]After standardization (culling).
*If there were more than 23 acceptable litters for any dietary level, the surplus litters were weighed and sacrificed without routine necropsy on PND 4.

2. **Body weight:** Body weight and body weight gain were comparable across all dose groups at birth and on PND 4. Body weight was decreased 8-11% in high-dose pups on subsequent days through weaning. Weight gain of high-dose pups was decreased 8.5%-13% from PND 4-21. After weaning, pups received only untreated diet, and the only compound-related effects were noted in high-dose males and females and were associated with the decreased body weight that developed during lactation. High-dose males weighed 10% less than controls during the first week post-weaning and 7% less during the last week of the study. High-dose females weighed 7.5% less than controls during the first week post-weaning and had weight comparable to controls by the last two weeks of the study. Sporadic statistical changes at lower doses were considered incidental to treatment. Selected mean pre-weaning pup body weight data are presented in Table 6, and selected mean post-weaning offspring body weight data are presented in Table 7.

TABLE 6. Mean (±SD) pre-weaning pup body weights and body weight gain (g) [a]

Postnatal Day	Dose (ppm)							
	0	30	125	200	0	30	125	200
	Males				Females			
N=	19	23	23	23	19	23	23	23
0	5.8±0.08	5.7±0.09	5.8±0.08	5.7±0.09	5.5±0.09	5.4±0.08	5.5±0.07	5.4±0.10
4 [b]	9.7±0.22	9.2±0.19	9.6±0.17	9.0±0.21	9.3±0.24	8.9±0.17	9.2±0.18	8.6±0.21
4 [c]	9.7±0.22	9.2±0.19	9.6±0.17	9.0±0.21	9.2±0.25	9.0±0.17	9.2±0.18	8.6±0.22
11	24.7±0.48	23.3±0.57	23.9±0.36	22.2**±0.51 (10%)	23.5±0.48	23.0±0.55	23.3±0.36	21.4*±0.54 (8.9%)
17	39.0±0.64	37.0±0.67	37.3±0.52	35.2**±0.72 (10%)	36.9±0.64	36.2±0.65	36.3±0.52	34.0*±0.75 (7.9%)
21	49.6±0.85	46.5*±0.79 (6.3%)	47.1±0.65	44.3**±0.83 (11%)	46.7±0.87	45.3±0.75	45.6±0.65	42.9**±0.86 (8.1%)
Weight gain Days 0-4	3.8±0.17	3.5±0.14	3.8±0.13	3.3±0.14	3.7±0.18	3.5±0.14	3.7±0.14	3.2±0.14
Weight gain Days 4-11	15.0±0.32	14.1±0.46	14.3±0.27	13.1**±0.37 (13%)	14.3±0.30	14.0±0.45	14.1±0.28	12.8**±0.39 (10%)
Weight gain Days 4-17	29.3±0.51	27.8±0.60	27.7±0.43	26.2**±0.62 (11%)	27.7±0.49	27.2±0.59	27.0±0.45	25.4*±0.64 (8.3%)
Weight gain Days 4-21	39.9±0.70	37.3*±0.69 (6.5%)	37.5*±0.55 (6.0%)	35.3**±0.72 (12%)	37.5±0.69	36.3±0.66	36.4±0.54	34.3**±0.72 (8.5%)

[a] Data obtained from Tables12-13, pages 81-89, MRID 46054101. *p≤0.05; **p≤0.01. Number in parentheses is % decrease compared to control, calculated by reviewer.
[b] Before standardization (culling).
[c] After standardization (culling).

18

TABLE 7 Mean (±SD) post-weaning pup body weights (g) [a]								
Postnatal Day (M/F)	Dose (ppm)							
	0	30	125	200	0	30	125	200
	Males				Females			
N=	19	23	23	23	19	23	23	23
29/30	77.5±8.1	75.6±7.1	72.6*±11.0 (6.3%)	69.5*±7.2 (10.3%)	76.8±8.8	76.6±7.9	73.5±9.9	71.0*±6.4 (7.5%)
36/37	124.3±10.3	120.8±9.4	118.1*±15.0 (4.9%)	113.3*±11.8 (8.8%)	112.1±10.0	110.0±10.1	107.8*±9.8	104.5*±7.3 (6.8%)
43/44	170.1±13.2	165.0±12.3	163.4*±16.0 (3.9%)	157.1*±15.8 (7.6%)	135.7±11.8	132.4±11.0	132.5±10.6	129.0*±8.7 (4.9%)
50/51	213.4±16.1	205.6*±15.5 (3.6%)	205.6*±18.2 (3.6%)	195.1*±20.8 (8.6%)	152.9±13.5	148.3±12.5	149.7±11.7	145..3*±10.1 (4.9%)
57/58	254.1±18.6	246.0±18.5	245.1±22.2	234.3*±25.0 (7.8%)	167.9±16.0	162.7±14.1	164.7±12.6	161.0*±10.9 (4.1%)
64/65	284.4±19.4	277.2±20.8	275.4±25.0	265.3*±25.9 (6.7%)	180.1±17.0	182.0±60.8	174.8±13.0	172.7±11.8
71/72	311.2±21.1	302.0±23.3	301.1±29.5	289.7*±26.9 (6.9%)	188.2±18.7	182.8±13.8	185.8±15.1	182.7±12.8

[a] Data obtained from Table15, pages 92-94, MRID 46054101. *p≤0.05. Number in parentheses is % decrease compared to control, calculated by reviewer.

There were no treatment-related effects on pre-weaning or post-weaning food consumption.

3. **Developmental landmarks:**

a. **Sexual maturation:** Preputial separation in males and mean age for attainment of vaginal opening for females were unaffected by treatment. The data are presented in Table 8.

TABLE 8. Mean (±SD) age of sexual maturation (days) [a]				
Parameter	Dose (ppm)			
	0	30	125	200
N (M/F)	57/57	69/69	65/69	66/69
Preputial separation (males)	43.6±0.34	43.9±0.29	43.8±0.32	44.2±0.35
Vaginal opening (females)	34.0±0.27	35.0*±0.25	34.4±0.23	34.6±0.24

[a] Data obtained from Table 14, pages 90-91, MRID 46054101. *p≤0.05

b. **Pupil constriction:** No treatment-related effects were noted. All control and treated rats exhibited pupil constriction on PND 21.

4. **Behavioral assessments:**

a. **Functional observational battery:** There were no significant treatment-related effects on offspring at any dose level on any test day (PND 4, 11, 21, 35, 45, or 60).

b. **Motor/locomotor activity:** No treatment-related overall or interval motor or locomotor activity effects were noted. For motor activity in control males and females, habituation was apparent on all four test days, including PND 13, when activity levels were relatively low. For locomotor activity, habituation was achieved on all test days, except PND 13 when activity was so low during the first interval for males and females that habituation was not evident. Total activity data are presented in Tables 9 and 10.

TABLE 9. Mean (±S.D.) motor activity data (total activity counts for session) [a]

Test Day	Dose (ppm)			
	0	30	125	200
Males				
PND 13	77±76	72±72	68±70	96±82
PND 17	253±143	174±119	224±151	225±94
PND 21	343±180	239±93	312±131	318±126
PND 60	572±142	549±102	586±145	523±107
Females				
PND 13	78±68	71±87	97±100	57±65
PND 17	196±90	257±181	263±127	194±153
PND 21	333±90	265±74	315±98	273±87
PND 60	669±126	788±147	727±139	774±209

[a] Data obtained from Table 19, pages 180-182, MRID 46054101.
N = 15-16/sex/dose.

TABLE 10. Mean (±S.D.) locomotor activity data (total activity counts for session) [a]				
Test Day	Dose (ppm)			
	0	30	125	200
Males				
PND 13	10±18	9±13	7±9	10±7
PND 17	62±28	39±26	53±40	58±34
PND 21	90±37	72±28	90±18	93±37
PND 60	399±124	363±73	407±116	363±102
Females				
PND 13	5±6	9±14	13±21	7±16
PND 17	47±22	71±62	73±49	53±56
PND 21	100±23	77±24	100±36	80±30
PND 60	432±102	523±109	454±113	519±158

[a] Data obtained from Table 20, pages 183-185, MRID 46054101. N = 15-16/sex/dose.

c. **Auditory startle reflex :** The only treatment-related effect was a 30% decrease in startle amplitude in high-dose males on PND 22. A statistically significant ($p < 0.05$) in peak mean amplitude auditory startle response was seen in males at the high dose. However, there was no dose-response. Therefore, the biological significance of this effect is not known at this time. A similar decrease was not seen in females. Peak amplitude data are summarized in Table 11 and latency data are summarized in Table 12.

TABLE 11. Auditory startle reflex peak amplitude data (mean g ±S.D.) [a]					
	Trial Block	Dose (ppm)			
		0	30	125	200
Males					
PND 22	1	53±21	43±16	46±16	43±15
	2	54±17	48±23	48±28	37±11
	3	54±15	41±24	47±25	39±16
	4	52±14	38±28	41±24	35±14
	5	50±20	33±21	38±25	31±12
	Mean	53±16	40±21	44±22	37*±12
PND 38	1	91±45	81±57	131±94	110±45
	2	115±69	69±44	131±123	107±67
	3	92±70	57±34	102±86	90±55
	4	77±51	55±36	102±87	89±64
	5	69±35	56±36	78±61	85±57
	Mean	89±48	64±37	109±85	96±53

TABLE 11. Auditory startle reflex peak amplitude data (mean g ±S.D.) [a]

	Trial Block	Dose (ppm)			
		0	30	125	200
PND 60	1	303±157	305±197	369±279	333±193
	2	299±135	269±202	348±262	338±247
	3	234±128	226±156	280±231	308±238
	4	148±91	160±114	185±164	232±171
	5	164±120	166±164	198±179	197±134
	Mean	230±115	225±158	276±212	282±189
Females					
PND 22	1	48±20	41±14	40±16	43±15
	2	49±20	41±15	39±14	45±20
	3	46±21	42±19	39±22	39±17
	4	41±22	38±14	35±19	35±18
	5	38±17	35±16	32±19	34±18
	Mean	44±19	39±15	37±17	39±16
PND 38	1	68±48	69±35	60±30	69±41
	2	56±38	55±38	56±32	55±33
	3	50±31	51±25	53±30	39±19
	4	54±40	50±28	43±15	37±26
	5	44±26	43±26	40±14	32±18
	Mean	54±33	54±26	50±19	47±25
PND 60	1	125±84	118±82	134±80	112±106
	2	107±59	106±100	125±71	94±117
	3	93±53	94±54	73±25	67±56
	4	79±48	78±41	77±38	58±40
	5	69±43	71±45	63±33	74±52
	Mean	95±50	93±56	94±39	81±71

[a]Data obtained from Tables 23-24, pages 204-213, MRID 46054101. *p≤0.05.
N = 16/sex/dose

TABLE 12. Auditory startle latency to peak data (mean msec ±S.D.) [a]

	Trial Block	Dose (ppm)			
		0	30	125	200
Males					
PND 22	1	40±9	42±8	43±10	41±10
	2	37±9	37±9	39±11	41±9
	3	35±9	36±9	38±10	37±9
	4	37±9	37±8	37±10	38±7
	5	37±8	39±9	41±13	36±6
	Mean	37±8	38±7	40±8	38±6
PND 38	1	36±6	37±6	37±6	36±6
	2	34±7	34±5	36±6	35±5
	3	34±6	36±6	38±8	35±7
	4	34±5	38±7	37±5	35±6
	5	33±6	35±7	38±6	35±9
	Mean	34±5	36±4	37±5	35±6
PND 60	1	38±3	38±5	38±4	36±3
	2	35±3	35±4	36±4	34±2
	3	36±5	36±6	36±3	34±4
	4	35±3	38±7	37±3	37±4
	5	36±5	36±5	37±6	36±5
	Mean	36±2	37±4	37±3	36±2
Females					
PND 22	1	40±6	45±9	39±7	44±12
	2	43±10	46±10	39±10	44±12
	3	38±6	42±11	40±9	45±13
	4	36±6	39±9	39±6	43±12
	5	37±6	42±10	38±10	41±10
	Mean	39±6	43±8	39±6	43±11
PND 38	1	38±7	37±5	37±5	38±6
	2	37±6	37±8	36±6	37±6
	3	38±7	37±6	37±5	40±7
	4	36±4	37±5	38±7	40±6
	5	40±6	37±6	36±6	40±7
	Mean	38±4	37±5	37±4	39±4
PND 60	1	42±5	44±5	41±5	43±5
	2	41±7	42±5	41±5	41±4
	3	41±6	41±6	42±8	44±6
	4	39±8	39±6	41±7	41±5

TABLE 12. Auditory startle latency to peak data (mean msec ±S.D.) [a]					
	Trial Block	Dose (ppm)			
		0	30	125	200
	5	42±6	41±7	40±6	45±4
	Mean	41±4	41±4	41±4	43±3

[a]Data obtained from Tables 23-24, pages 204-213, MRID 46054101.
N = 16/sex/dose

d. Learning and memory testing:

Passive Avoidance: There were no treatment-related effects, and acquisition and retention were appropriate in control animals. Data are summarized in Table 13.

TABLE 13. Passive avoidance performance at PND 24/31(mean ± S.D.) [a]					
Test Day/Parameter		Dose (ppm)			
		0	30	125	200
		Males			
Session 1 (Learning)	Trials to criterion	2.9±0.3	3.1±0.6	3.2±0.5	3.1±0.3
	Latency trial 1 (sec)	50.5±41.4	44.7±51.4	29.4±23.2	25.9±22.4
	Latency trial 2 (sec)	180.0±0.0	169.4±42.4	178.0±8.0	175.9±14.0
	Failed to Learn/No. Tested	1/16	1/16	0/16	0/16
Session 2 (Retention)	Trials to criterion	2.1±0.4	2.1±0.3	2.0±0.0	2.1±0.5
	Latency trial 1 (sec)	172.5±21.7	176.0±15.4	180.0±0.0	180.0±0.0
	Latency trial 2 (sec)	180.0±0.0	180.0±0.0	180.0±0.0	179.5±1.9
		Females			
Session 1 (Learning)	Trials to criterion	3.0±0.0	3.1±0.8	3.0±0.0	3.1±0.3
	Latency trial 1 (sec)	34.9±19.8	45.1±48.2	26.3±25.4	31.6±28.2
	Latency trial 2 (sec)	180.0±0.0	171.7±33.3	180.0±0.0	174.5±21.8
	Failed to Learn/No. Tested	0/16	1/16	0/16	0/16
Session 2 (Retention)	Trials to criterion	2.1±0.3	2.1±0.5	2.2±0.5	2.3±0.6
	Latency trial 1 (sec)	168.7±38.3	180.0±0.0	170.4±38.4	166.1±40.5
	Latency trial 2 (sec)	180.0±0.0	169.9±39.0	179.0±3.8	173.8±25.0

[a] Data obtained from Table 25, pages 214-216, MRID 46054101.

Water Maze: Data are summarized in Table 14. There were no treatment-related differences for males or females at any dose level, compared to controls, with regard to trials to criterion, time to escape, number of errors, or failure to meet criterion.

24

TABLE 14. Water maze performance

Test Day/Parameter		Dose (ppm)			
		0	30	125	200
Males					
Session 1 (Learning)	Trials to criterion	6.8±2.2	6.2±1.6	7.9±3.2	9.0±3.8
	Trial 1 errors (mean ± SD)	0.8±0.9	0.7±0.9	0.7±0.8	1.0±1.1
	Trial 1 duration (sec) (mean ± SD)	19.9±15.3	23.3±17.2	18.5±13.3	29.5±22.4
	Trial 2 errors (mean ± SD)	0.5±1.0	0.1±0.5	0.5±0.7	1.0±1.3
	Trial 2 duration (sec) (mean ± SD)	13.2±12.5	7.8±4.3	14.1±11.9	22.4±19.7
	Failed to meet criterion	0/16 (0%)	0/16 (0%)	1/15 (7%)	1/15 (7%)
Session 2 (retention)	Trials to criterion	5.4±0.5	5.0*±0.0	5.6±2.1	5.5±1.1
	Trial 1 errors (mean ± SD)	0.7±1.1	0.0*±0.0	0.1±0.4	0.4±0.6
	Trial 1 duration (sec) (mean ± SD)	10.6±10.1	4.7±2.4	6.6±4.4	8.9±5.8
	Trial 2 errors (mean ± SD)	0.0±0.0	0.0±0.0	0.0±0.0	0.1±0.5
	Trial 2 duration (sec) (mean ± SD)	3.8±1.7	3.1±0.6	3.9±1.7	6.1±10.3
Females					
Session 1 (Learning)	Trials to criterion	6.4±1.7	8.8±3.4	7.4±3.2	6.6±2.3
	Trial 1 errors (mean ± SD)	0.3±0.4	0.6±0.9	0.8±1.2	0.6±0.6
	Trial 1 duration (sec) (mean ± SD)	9.9±6.4	13.5±7.7	16.6±14.5	16.4±12.6
	Trial 2 errors (mean ± SD)	0.6±0.9	0.4±0.7	0.6±1.1	0.6±0.8
	Trial 2 duration (sec) (mean ± SD)	12.4±8.8	9.7±4.1	13.6±15.0	14.2±11.1
	Failed to meet criterion	0/16 (0%)	2/16 (13%)	2/16 (13%)	0/16 (0%)
Session 2 (retention)	Trials to criterion	5.8±1.4	5.9±1.5	7.9±3.1	5.9±1.6
	Trial 1 errors (mean ± SD)	0.3±0.7	0.3±0.6	0.3±0.6	0.1±0.3
	Trial 1 duration (sec) (mean ± SD)	10.2±11.2	8.9±7.6	8.6±6.2	6.6±5.3
	Trial 2 errors (mean ± SD)	0.1±0.3	0.0±0.0	0.1±0.4	0.0±0.0
	Trial 2 duration (sec) (mean ± SD)	3.9±1.7	4.9±3.5	5.3±6.1	4.0±1.3

[a]Data obtained from Table 26, pages 217-219, MRID 46054101. *p≤0.05.

e. **Ophthalmology:** There were no treatment-related ocular effects in any treated animals compared to controls.

25

5. Postmortem results:

a. **Brain weight:** Mean brain weight data are presented in Table 15. On PND 21, no treatment-related effects on absolute or relative brain weight were noted for males or females in any group. At study termination, absolute and relative brain weights were not affected by treatment in males in any dose group. Absolute brain weight was decreased 5.8% ($p \leq 0.05$) in high-dose females compared to controls.

TABLE 15. Mean (±SD) Brain Weight Data in Offspring [a]

Parameter	Dose (ppm)			
	0	30	125	200
Males				
Day 21				
Terminal body weight (g)	49.1±3.9	45.8±5.2	46.6±3.2	44.9±2.7
Brain weight (g)	1.420±0.061	1.401±0.062	1.420±0.049	1.416±0.054
Brain-to-body weight ratio	2.902±0.178	3.089±0.308	3.055±0.168	3.164±0.192
Termination				
Terminal body weight (g)	319.2±22.7	306.7±26.2	307.6±26.8	297.6±14.1
Brain weight (g)	1.880±0.061	1.863±0.064	1.834±0.073	1.832±0.069
Brain-to-body weight ratio	0.591±0.037	0.611±0.045	0.601±0.064	0.616±0.028
Females				
Day 21				
Terminal body weight (g)	47.1±3.8	44.7±4.0	46.2±4.4	44.8±5.1
Brain weight (g)	1.404±0.040	1.388±0.059	1.397±0.052	1.342±0.059
Brain-to-body weight ratio	2.996±0.250	3.127±0.329	3.043±0.262	3.027±0.332
Termination				
Terminal body weight (g)	197.5±16.5	184.5±10.9	186.9±10.1	180.7*±12.2 (8.5%)
Brain weight (g)	1.757±0.052	1.731±0.055	1.719±0.051	1.655*±0.100 (5.8%)
Brain-to-body weight ratio	0.895±0.073	0.942±0.066	0.922±0.063	0.920±0.089

[a] Data obtained from pages 916-921, MRID 46054101. *$p \leq 0.05$. Number in parentheses is % decrease compared to control, calculated by reviewer.
N = 9-10/sex/dose

b. β-Cyfluthrin concentration in brain tissue: β-Cyfluthrin was detected in a dose-related manner in brain tissue from pups in all treatment groups on PND 4 and PND 21. Data are summarized in Table 16.

TABLE 16. Concentration (ppm) of β-Cyfluthrin in whole brain tissue of pups

	Dose (ppm)			
	0	30	125	200
PND 4	0.000	0.004	0.016	0.026
PND 21	0.002	0.006	0.024	0.034

Data obtained from page 49, MRID 46054101.

c. Neuropathology

1. **Macroscopic examination**: No treatment-related effects were reported for male or female offspring at postnatal day 21 or study termination.

2. **Microscopic examination**: No significant treatment-related effects were noted on postnatal day 21 or study termination.

3. **Brain Morphometry:** No treatment-related morphometric effects were observed in any animals at PND 21 or study termination. Data are summarized in Table 17.

TABLE 17. Mean (±SD) morphometric data in offspring [a]				
Parameter	Dose (ppm)			
	0	30	125	200
Males				
Day 21				
Anterior to posterior cerebrum length (mm)	13.34±0.28	13.00±0.24	13.27±0.28	13.18±0.34
Anterior to posterior cerebellum length (mm)	6.90±0.39	6.74±0.32	6.67±0.33	7.06±0.30
Termination				
Anterior to posterior cerebrum length (mm)	14.83±0.31	14.63±0.28	14.71±0.32	14.55±0.33
Anterior to posterior cerebellum length (mm)	7.78±0.34	7.69±0.34	7.83±0.39	7.90±0.44
Females				
Day 21				
Anterior to posterior cerebrum length (mm)	13.18±0.24	12.98±0.42	13.03±0.37	13.06±0.39
Anterior to posterior cerebellum length (mm)	6.82±0.37	6.94±0.27	6.81±0.31	6.89±0.42
Termination				
Anterior to posterior cerebrum length (mm)	14.38±0.41	14.29±0.24	14.18±0.30	14.16±0.38
Anterior to posterior cerebellum length (mm)	7.92±0.19	7.97±0.43	7.92±0.33	7.72±0.33

[a] Data obtained from pages 916-921, MRID 46054101.
N = 9-10/sex/dose

III. DISCUSSION and CONCLUSIONS:

A. **INVESTIGATORS' CONCLUSIONS:** The investigators concluded that the NOAEL is 125 ppm and the LOAEL is 200 ppm for dams based on decreased body weight and food consumption at 200 ppm. The investigators also concluded that the NOAEL for offspring is 125 ppm and the LOAEL is 200 ppm based on decreased body weights in males and females in the 200 ppm group during lactation and after weaning.

B. **REVIEWER COMMENTS:** In dams, no treatment-related effects on mortality, clinical signs, body temperature, or FOB parameters were noted. Body weight was decreased 7% ($p \leq 0.01$) in high-dose dams during the first week of exposure; however, there were no differences in the subsequent weeks of gestation. During lactation, body weight was decreased in high-dose dams on LD 4 (8%), LD 7 (6%), and LD 14 (7%), but was comparable to controls on LD 21. These decreases were not considered to be adverse because they were transient, the decrease was of low magnitude (7-8%), occurred in the absence of any effect on food consumption, and rebounded on LD21. Additionally, there

were no other treatment-related effects on body weight or body weight gain during gestation or lactation.

In offspring, there were no treatment-related deaths or clinical signs or effects on birth weight, developmental landmarks, FOB parameters, motor or locomotor activity, passive avoidance, learning and memory, or opthalmological parameters. Body weight and body weight gain were comparable across all dose groups at birth and on PND 4. Body weight was decreased 8-11% in high-dose pups on subsequent days through weaning. Weight gain of high-dose pups was decreased 8.5%-13% from PND 4-21. After weaning, pups received only untreated diet, and the only compound-related effects were noted in high-dose males and females and were associated with the decreased body weight that developed during lactation. High-dose males weighed 10% less than controls during the first week post-weaning and 7% less during the last week of the study. High-dose females weighed 7.5% less than controls during the first week post-weaning and had weight comparable to controls by the last two weeks of the study. A statistically significant ($p < 0.05$) decrease (30%) in peak mean amplitude auditory startle response was seen in males at the high dose on PND 22. However, there was no dose-response. Therefore, the biological significance of this effect is not known at this time. A similar decrease was not seen in females. At necropsy, absolute brain weight was decreased 5.8% ($p \leq 0.05$) in high-dose females compared to controls. There were no treatment-related gross or micropathological lesions or changes in brain morphology were seen. .

The maternal NOAEL is 17.8 mg/kg/day, the highest dose tested. A maternal LOAEL was not established.

The offspring LOAEL is 17.8 mg/kg/day based on decreased body weight and body weight gain and decreased brain weights in females at termination. The offspring NOAEL is 11.0 mg/kg/day.

This study is classified **Acceptable/non-guideline** and may be used for regulatory purposes; however, it does not satisfy the guideline requirement for a developmental neurotoxicity study in rats (OPPTS 870.6300, §83-6; OECD 426 (draft)) at this time pending a comprehensive review of all available positive control data.

C. **STUDY DEFICIENCIES:** None

13544

R158226

Chemical:	**beta-cyfluthrin** **Cyfluthrin**
	PC Code: **118831** **128831**
HED File Code:	**11100 Other Chemistry Documents**
Memo Date:	**9/6/2007**
File ID:	**TX0052372**
Accession #:	**000-00-0124**

HED Records Reference Center
3/31/2008